

We claim:

1. A method for amplifying a signal from a binding assay comprising the steps of:  
providing a reaction mixture comprising in combination:

5           a medium suspected of containing an analyte;  
          a first specific binding pair member associated with a support;  
          a second specific binding pair member associated with a sensitizer capable  
in its excited state of generating a reactive oxygen species, wherein the association of first  
specific binding pair member with the second specific binding pair member is modulated  
10 by the presence of the analyte; and  
          a detectable substrate comprising a ligand associated with the support  
through a reactive oxygen cleavable linker;  
          incubating the reaction mixture to allow association of the first and second specific  
binding pair members;  
15        exciting the sensitizer, said excitation of the sensitizer causing the release of  
detectable substrate from the support; and  
          detecting the released detectable substrate.

2. The method of claim 1 wherein:

20           the association of the first and second specific binding pair members results  
from the binding of the first and second specific binding pair members to the analyte;  
          the sensitizer is a photosensitizer;  
          the reactive oxygen species is singlet oxygen; and  
          the excitation step comprises irradiation of the photosensitizer with light.

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3. The method of claim 2 wherein:

          the analyte, first specific binding pair member, and second specific binding pair  
member are polynucleotides;  
          the substrate comprises digoxigenin-linked biotin; and  
30        the step of detecting the released detectable substrate is carried out by a detection  
method comprising avidin and anti-digoxigenin antibodies, wherein the detection method

utilizes a technology selected from the group comprising LOCI, FOCI, ELISA, RIA, and FETI.

4. The method of claim 2 wherein the reactive oxygen cleavable linker comprises an olefin or an aromatic compound.
5. The method of claim 4 wherein said olefin is selected from the group consisting of dioxenes, thioxenes, oxazines, dithienes, thioenolethers, enolethers, and enamines.
6. The method of claim 4 wherein said aromatic compound is selected from the group consisting of oxazoles, thiazoles, imidazoles, naphthalenes, anthracenes and diacylhydrazides.
7. The method of claim 1 wherein:
  - the reactive oxygen species is hydrogen peroxide or singlet oxygen; and
  - the sensitizer comprises an enzyme or an electron transfer catalyst.
8. The method of claim 7 wherein the reactive oxygen species is hydrogen peroxide and the reactive oxygen cleavable link comprises a group selected from disulfides, alkylborons, p-hydroxyphenyl ether, and p-aminophenyl ether.
9. The method of claim 1 wherein the step of detecting the released detectable substrate comprises the steps of:
  - separating the released detectable substrate from the detectable substrate associated with the support;
  - adding to the separated released detectable substrate, a third specific binding pair member capable of binding directly or indirectly to the released detectable substrate;
  - allowing the third specific binding pair member to bind to the released detectable substrate; and
  - detecting the bound third specific binding pair member.

10. The method of claim 1 wherein the step of detecting the released detectable substrate comprises the steps of:

including a third specific binding pair member, and a fourth specific binding pair member, wherein the third specific binding pair member is capable of associating with the fourth specific binding pair member in the presence of the released detectable substrate;

incubating the reaction mixture to allow association of the third and fourth specific binding pair members; and

detecting the associated third and fourth specific binding pair members.

11. The method according to claim 10 further comprising separating the released detectable substrate from the detectable substrate associated with the support.

12. The method of claim 10 wherein the third specific binding pair member comprises avidin and the fourth specific binding pair member comprises an anti-digoxigenin antibody.

13. The method of claim 1 wherein the step of detecting the released detectable substrate comprises the steps of:

adding to the separated released detectable substrate in combination a third specific binding pair member capable of binding directly or indirectly to a first binding site, said first binding site being found on the released detectable substrate; and a free labeled competitor containing the first binding site;

incubating the reaction mixture to allow competitive binding of the released detectable substrate and the labeled competitor to the third specific binding pair member, forming a bound detectable substrate or a bound labeled competitor; and

detecting the free labeled competitor or the bound labeled competitor, wherein said detection is related to the amount of analyte in the medium suspected of containing the analyte.

14. The method according to claim 13 further comprising separating the released detectable substrate from the substrate.

15. The method of claim 1 wherein said detection of the released detectable substrate comprises detecting a first unmasked binding site on the released detectable substrate, said first unmasked binding site being masked when said detectable substrate is associated with the support.

16. The method of claim 11 wherein the sensitizer is a photosensitizer, the reactive oxygen species is singlet oxygen; and the excitation step comprises irradiation of the photosensitizer with light.

17. The method of claim 16 wherein the analyte, first specific binding pair member, and second specific binding pair member are polynucleotides, the substrate comprises digoxigenin-linked-biotin, the step of detecting the released detectable substrate is carried out by a detection method comprising avidin and anti-digoxigenin antibodies, wherein the detection method utilizes a technology selected from the group comprising LOCI, FOCI, ELISA, RIA, and FETI.

18. The method of claim 16 wherein the reactive oxygen cleavable linker comprises an olefin or an aromatic compound.

19. The method of claim 18 wherein said olefin is selected from the group consisting of dioxenes, thioxenes, oxazines, dithienes, thioethers, enolethers, and enamines.

20. The method of claim 18 wherein said aromatic compound is selected from the group consisting of oxazoles, thiazoles, imidazoles, naphthalenes, anthracenes and diacylhydrazides.

21. The method of claim 15 wherein the reactive oxygen species is hydrogen peroxide or singlet oxygen and the sensitizer comprises an enzyme or an electron transfer catalyst.

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the reagent of claim 25; and

a second specific binding pair member associated with a sensitizer capable in its excited state of generating a reactive oxygen species, wherein the first specific binding pair member is capable of associating with the second specific binding pair member in the presence of the analyte.

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27. A kit for determining the presence of an analyte, comprising:

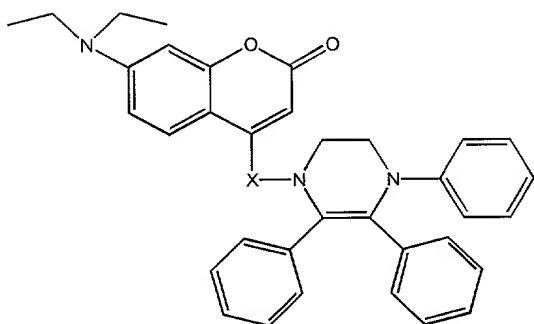
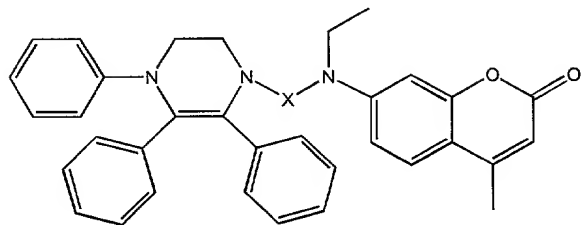
a composition comprising a singlet oxygen cleavable linker; and  
a photosensitizer capable in its excited state of generating singlet oxygen.

10 28. A composition comprising a singlet oxygen activatable indicator precursor  
compound of the general formula F-Q, wherein F is a fluorescer moiety capable of  
being induced to fluoresce by light at wavelengths less than 550 nm and Q is a  
quencher, wherein F and Q are linked such that Q quenches F and wherein Q is  
capable of reaction with singlet oxygen said reaction inactivating the quenching  
15 property of Q and producing a fluorescent indicator compound.

29. The composition of claim 28 wherein said wavelength is around 400 nm.

20 30. The composition of claim 28 wherein the quencher moiety Q is selected from the  
group consisting of hydrazines, hydroxylamines, enol ethers, enol thioethers, and  
enamines.

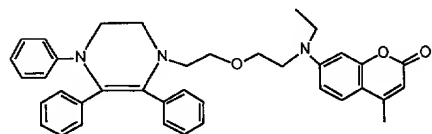
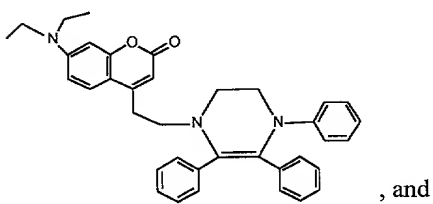
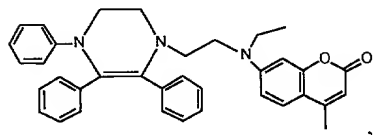
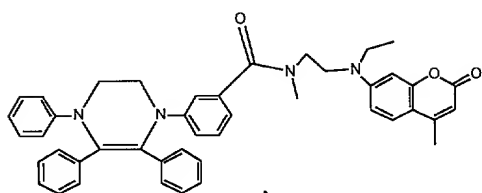
31. The composition of claim 28 wherein F-Q is selected from the group consisting of



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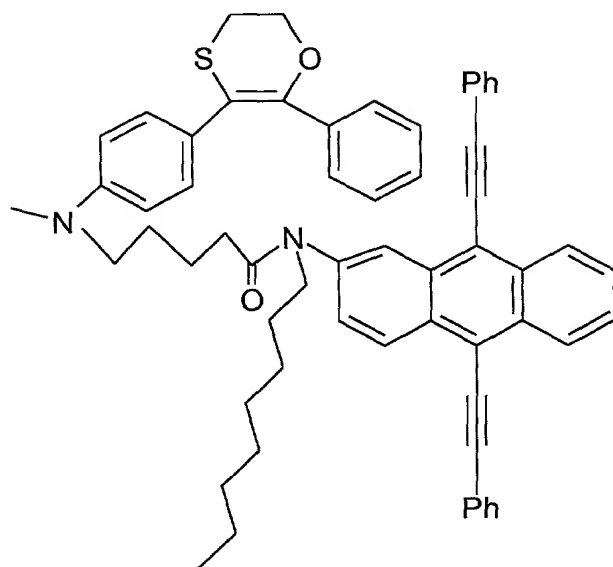
wherein X represents  $-\text{CONR}(\text{CH}_2)_n-$ ,  $-(\text{CH}_2)_n-$ ,  $-\text{aryl-CONR}(\text{CH}_2)_n-$  or  $-\text{aryl-CONR}(\text{CH}_2)_n-$ ; n represents 1 to 5; and aryl represents o-, m-, or p-phenyl.

32. The composition of claim 31 wherein F-Q is selected from the group consisting of





33. The composition of claim 28 wherein F-Q is:



34. The composition of claim 28 wherein Q is L-Q', wherein F and Q' are linked by a singlet oxygen cleavable linker L such that reaction of singlet oxygen with L separates Q' from F, thereby producing a fluorescent indicator compound.

35. The composition of claim 34 wherein L is selected from the group consisting of anthracenes, oxazoles, furans, hydrazides, and olefins.

36. The composition of claim 34 wherein Q' is selected from the group consisting of aliphatic amines, aromatic amines, phosphines, hydroquinones, phenolates, polyalkoxyaromatic compounds, hydrazines, hydroxylamines, quinones, alpha-diketones, polycyano aromatic compounds, polynitro aromatic compounds, phthalimide, and diazenes (azo compounds).